KARYOTYPE RELATIONSHIPS OF NATIVE NEW WORLD *VICIA* SPECIES (LEGUMINOSAE)[1]

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Vicia (Leguminosae: Paplionoideae) with an estimated 140 to over 170 species (Plitmann, 1967; Gunn & Kluve, 1976; Kupicha, 1976) is a world wide temperate zone genus of tendril-bearing vines. This genus exhibits great karyotypic diversity that is significant at the species level (Sveshnikova, 1927; Heitz, 1931; Shrivistava, 1963; Mettin & Hanelt, 1968; Stankevich, 1970; Yamamoto, 1973; Lassetter, 1975; and many others, list not inclusive). Except for a few reports (Rousi, 1961; Mettin and Hanelt, 1968; Lassetter, 1972, 1975; Veerasethakul, 1978; Veerasethakul & Lassetter, 1979; Lassetter & Gunn, 1979), the karotype work has been restricted to species native to the Old World.

A mixture of native and naturalized vetches occurs in the New World. In North America only about 15 or 16 of the 35 species are native (Hermann, 1960; Gunn, 1971; also see Rousi, 1961, and Veerasethakul & Lassetter, 1979, concerning native status of *Vicia cracca*). In Central America (including Mexico), 6 to 10 species are native New World taxa (Gunn, 1979). No comprehensive treatment of South American vetches exists, and the status of several species there is questionable (see Lassetter & Gunn, 1979, for taxonomy of *V. nigricans*). Probably about 20 "good" species occur in South America.

Native North American species listed in Hermann (1960) for which we had no seed material are *Vicia reverchonii* (probably now extinct) and *V. hugeri* (rare piedmont taxon in the southeastern U.S.). The species status is questionable for both taxa. We also had no seeds of *V. pulchella* or *V. leucophaea*. From Central America and Mexico, we studied four of the six native species. Our South American seeds were very limited, and we could include only *V. nigricans* and *V. graminea*. The species for which we do present karyotypes represent about 35% of the native New World vetches, and all belong to the subgenus *Vicilla* sensu Kupicha (1976).

This report presents karyotypes of native New World *Vicia*

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[1]The information presented here represents a Master's thesis submitted by the senior author to Eastern Kentucky University, 1978, as well as additional contributions by the junior author.

species, and groups of species based on karyotypes are compared with Kupicha's subgeneric groupings. Kupicha (1976) has presented the most recent major taxonomic treatment of the genus, but she did not use karyotypes as taxonomic characters in delimiting subgeneric taxa.

MATERIALS AND METHODS

Where possible, seed samples were obtained from several portions of the ranges of the species. Specific sources and identifications, collection sites, and location of population vouchers from which seeds were taken are given in the figure captions. In those captions, BARC indicated the seeds were obtained from the seed collection of the Agricultural Research Center, Beltsville, Maryland. Samples with DAO were provided by the Biosystematics Research Institute, Ottawa, Canada. Herbarium acronyms used here and elsewhere follow Holmgren and Keuken (1974). Seeds identified with C. R. Gunn's collection numbers were provided by Gunn from his personal collection, but population vouchers are in NA. Lassetter's collection numbers are represented by vouchers in ISC and EKU (Eastern Kentucky University). Other seeds were taken from the indicated herbarium sheets at the respective herbaria.

Seed coats were nicked with a razor blade, seeds germinated in rolled wet paper, and seedlings were transferred to pots of perlite and fertilized until root tips were removed for squashes. Adequate dividing cells were found from 1 to several hours after seedlings received morning sunlight. After root tips were removed, seedlings were transplanted to a mixture of one part peat, one part perlite, and three parts potting soil, and were grown as chromosome vouchers. Most of these vouchers are in EKU but a few are in ISC.

The root tips were pretreated with 0.002M 8-hydroxyquinoline for 4 hours in a 13° C water bath. The tips were fixed in 3:1 (V/V) absolute ethanol:glacial acetic acid for 15 minutes at 60° C, and hydrolyzed for 10 minutes in 1N HCl at 60° C. They were then Feulgen stained with Schiff's reagent for 1 hour. The tips were squashed in 45% acetic acid. All slides were made permanent by the CO_2 freezing method (Bowen, 1956) using Euparal as a mounting medium.

Most squash preparations were examined on a Bausch and Lomb stereozoom compound microscope with a 100× oil immersion

Plate 1. Figures **1–3**. *V. gigantea* Hook. 1. USDA *Barclay 1664* Clallam Co., Washington, USA BARC 2. USDA *Terrell s.n.* Lincoln Co., Oregon, USA BARC 3. C. R. *Gunn 3670* Clallam Co., Washington, USA NA. Figures **4–7**. *V. nigricans* Hooker & Arnott 4. USDA *PI 349242* Nahuel Huapi, Argentina BARC 5. USDA *PI 349244* Lawin, Argentina BARC 6. USDA *PI 349244* Pts. Mawzawo, Argentina BARC 7. USDA *PI 349243* Near Puerto, Argentina BARC

objective, and drawings of karyotypes were made from permanently mounted slides using a Zeiss camera lucida. Other slides were examined on a Leitz Laborlux microscope with a 90× apochromatic oil immersion objective, and drawn using a Leitz drawing apparatus.

RESULTS AND DISCUSSION

The species of this study have been categorized into three groups based on karyotype appearance.

Species in group one (Plate 1) contained the largest chromosomes of the species studied. These two species and *Vicia menziesii* of Hawaii form a trio of vetches which share several unusual *Vicia* traits (Lassetter & Gunn, 1979). These three species belong to Kupicha's Section *Cassubicae*, although she did not list *V. gigantea* (indicating her unproposed taxonomic position that *V. gigantea* and *V. nigricans* are conspecific) or *V. menziesii* (previously believed extinct).

Group two (Plate 2) contains species with distinctive karyotypes containing chromosomes with secondary constrictions near the centromere and no satellited chromosomes. The chromosomes of these species are all about the same size except for those of *Vicia graminea*, which are slightly smaller. Kupicha (1976) placed *V. americana* in the monotypic section *Americanae*, but she did not include *V. hassei* (Lassetter, 1975) — with two chromosome pairs with secondary constrictions near the centromere, or *V. humilis* in her study. These two species are listed by Gunn (1979) as members of section *Cracca*. *Vicia graminea* was placed in Section *Australes* by Kupicha, who was able to study more South American species than we. The karyotype of *V. graminea* does resemble karotypes of the other members of group two, but it should be compared with karyotypes of other South American species. The karyotype of *V. graminea* compares favorably with the one presented by Mettin and Hanelt (1968).

This group of species, while sharing karyotype similarities, is diverse in other characteristics, and contains representatives of three different sections sensu kupicha (1976).

Group three (Plates 3, 4, 5, & 6) has smaller chromosomes with satellites and no secondary constrictions near the centromere. All the taxa of this group are placed in Section *Cracca* by Kupicha (1976). Our samples of *Vicia cracca* (Plate 3) were tetraploid, but the basic diploid set is like the diploid species of this group. Rousi (1961) and Veerasethakul and Lassetter (1979) have discussed geographical distribution and ploidy in *V. cracca*.

The Vicia ludoviciana complex (Plate 4), including *V. ludoviciana*, *V. leavenworthii*, *V. exigua*, and their varieties, was the subject of

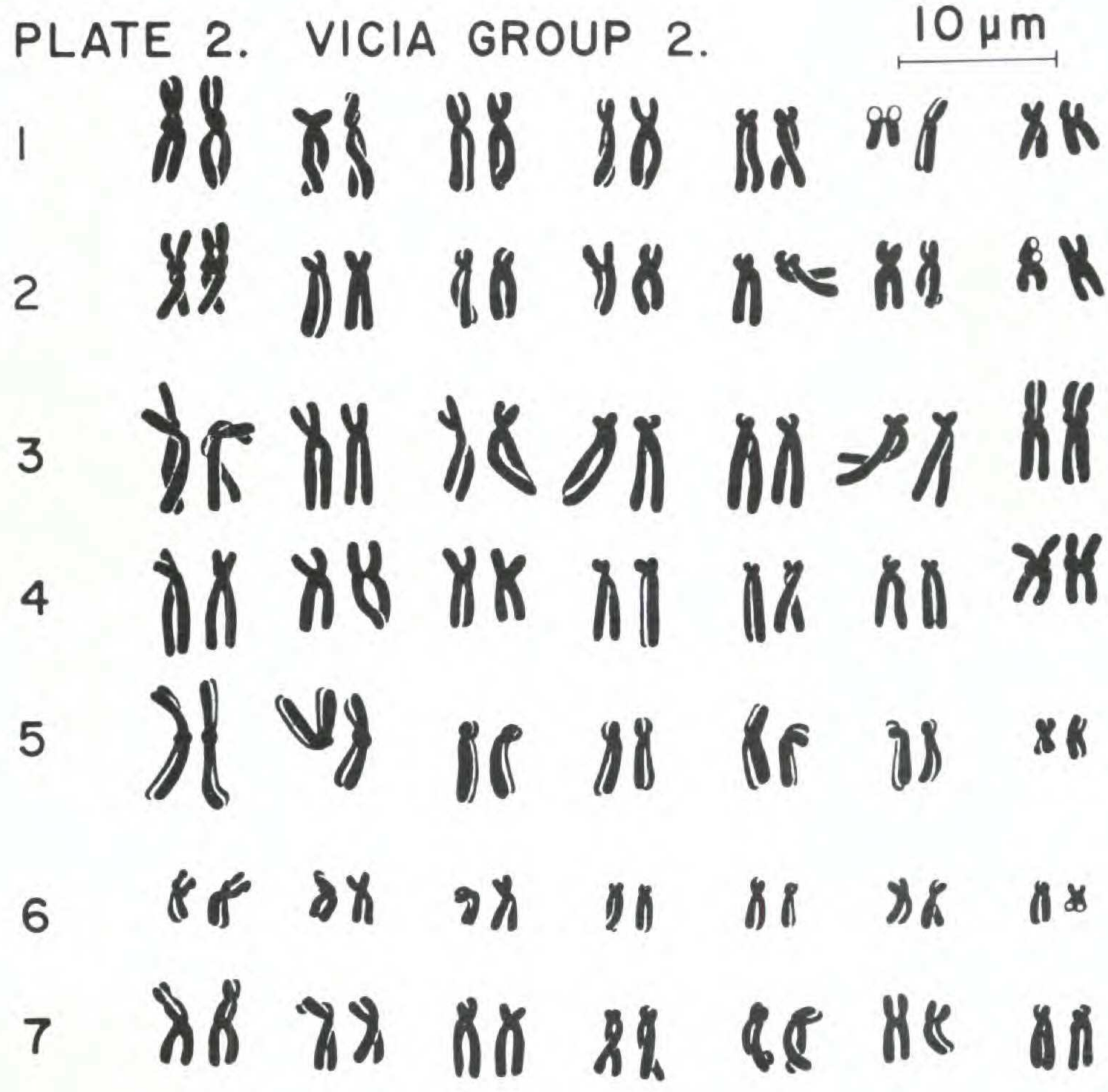

Plate 2. Figure **1 & 2.** *V. americana* Muhl. 1. *C. R. Gunn 2631* Riverside Co., California, USA NA 2. *C. R. Gunn 2566* Story Co., Iowa, USA ISC Figures **3 & 4.** *V. humilis* HBK. 3. USDA *J. Rzedowski 24058* Coatlinchan, Mexico BARC 4. USDA *PI 343008* Morelos, Mexico BARC Figure **5.** *V. hassei* S. Wats. 5. *Wiggins and Ernst 123* Guadalupe Island, Baja California, Mexico DS Figure **6 & 7.** *V. graminea* Sm. 6. USDA *L. G. Labouriau s.n.* Sao Paulo, Brazil BARC 7. USDA *DEIP 20.258* Buenos Aires, Argentina BARC

biosystematic study (Lassetter, 1972, 1975, 1978 a&b, and in press). The complex was reevaluated as one wide ranging species, *V. ludoviciana,* with one subspecies of five geographical races and a second subspecies of two races. The kind of infraspecific karyotype variation exhibited by these taxa is similar to that shown by Rousi (1961) to exist in *V. tenuifolia.* Kupicha (1976) viewed *V. ludoviciana,, V. leavenworthii,* and *V. exigua* as separate species, but was not aware of the recent work on the complex.

PLATE 3. VICIA GROUP 3.

Plate 3. Figures **1–6**. *V. cracca* L. 1. DAO *W. E. Kemp & T. F. Adams s.n.* Ontario, Canada DAO 2. *C. R. Gunn 3768* Meductic, New Brunswick, Canada NA 3. USDA *NJ-64-68* Cape May Co., New Jersey, USA BARC 4. *C. R. Gunn 3770* Quebec, Canada NA 5. USDA *PI 234266* Ontario Co., New York, USA BARC 6. *C. R. Gunn 3759* Cumberland Co., Nova Scotia, Canada NA

PLATE 4. VICIA GROUP 3. 10 µm

Plate 4. Figure 1. *V. ludoviciana* Nutt. subspecies *ludoviciana* race 1 *Lassetter 1753* Fayette Co., Texas, USA ISC Figure 2. *V. ludoviciana* Nutt. subspecies *ludoviciana* race 5 *Lang 4665* Menard Co., Texas, USA ISC Figure 3. *V. ludoviciana* Nutt. subspecies *ludoviciana* race 2 *Lassetter 1773* Aransas Co., Texas, USA ISC Figure 4. *V. leavenworthii* Nutt. subspecies *ludoviciana* race 7 *Goodman and Lawson 8171* McCurtain Co., Oklahoma, USA OKL Figure 5. *V. ludoviciana* Nutt. subspecies *leavenworthii* race 6 *Lassetter 1833* Dallas Co., Texas, USA ISC Figure 6. *V. ludoviciana* Nutt. subspecies *ludoviciana* race 4 *Lassetter 1731* Culberson Co., Texas, USA ISC Figure 7. *V. ludoviciana* Nutt. subspecies *ludoviciana* race 3 *I. Marin, s.n.* Riverside Co., California, USA ISC

Vicia caroliniana and *V. minutiflora* (Plate 5) have karyotypes similar to those of the *V. ludoviciana* complex, but satellites are located on different chromosomes and centromere placement is different. The size range is comparable.

The three species of Plate 6 are a related trio, and *Vicia acutifolia* and *V. floridana* have been recognized as valid species since their naming in the 1800's. *Vicia ocalensis,* endemic to Florida and not

Plate 5. Figures **1–4.** *V. caroliniana* Walt. 1. USDA *K. E. Rogers & G. Morton s.n.* Blount Co., Tennessee, USA BARC 2. USDA *Bartholomew s.n.* Wirt Co., West Virginia, USA BARC 3. *Lassetter 2523* Garrard Co., Kentucky, USA BARC 4. *C. R. Gunn 3634* Macon Co., North Carolina, USA NA **5–6.** *V. minutiflora* Dietr. 5. *L. H. Shinners 10899* Henderson Co., Texas, USA SMU 6. USDA *C. E. Smith, Jr. 5039* Hale Co., Alabama, USA BARC

studied by Kupicha, was named as a new species by Godfrey and Kral (1958). On morphological characteristics, Godfrey and Kral as well as Hermann (1960) showed that *V. ocalensis* resembles *V. acutifolia* more than *V. floridana.* Our karyotype data also support this relationship.

All these section *Cracca* species of group three share a basically similar karyotype. The chromosomes are about the same size from species to species, and each species has one satellited pair, except for *Vicia ocalensis* which has two satellited pairs.

Section *Cracca* is the largest one in Kupich's treatment and she stated it probably could be subdivided. We, on the basis of karyotype data alone, do not suggest a division, but if *Vicia hassei* and *V. humilis* are valid members of section *Cracca,* other

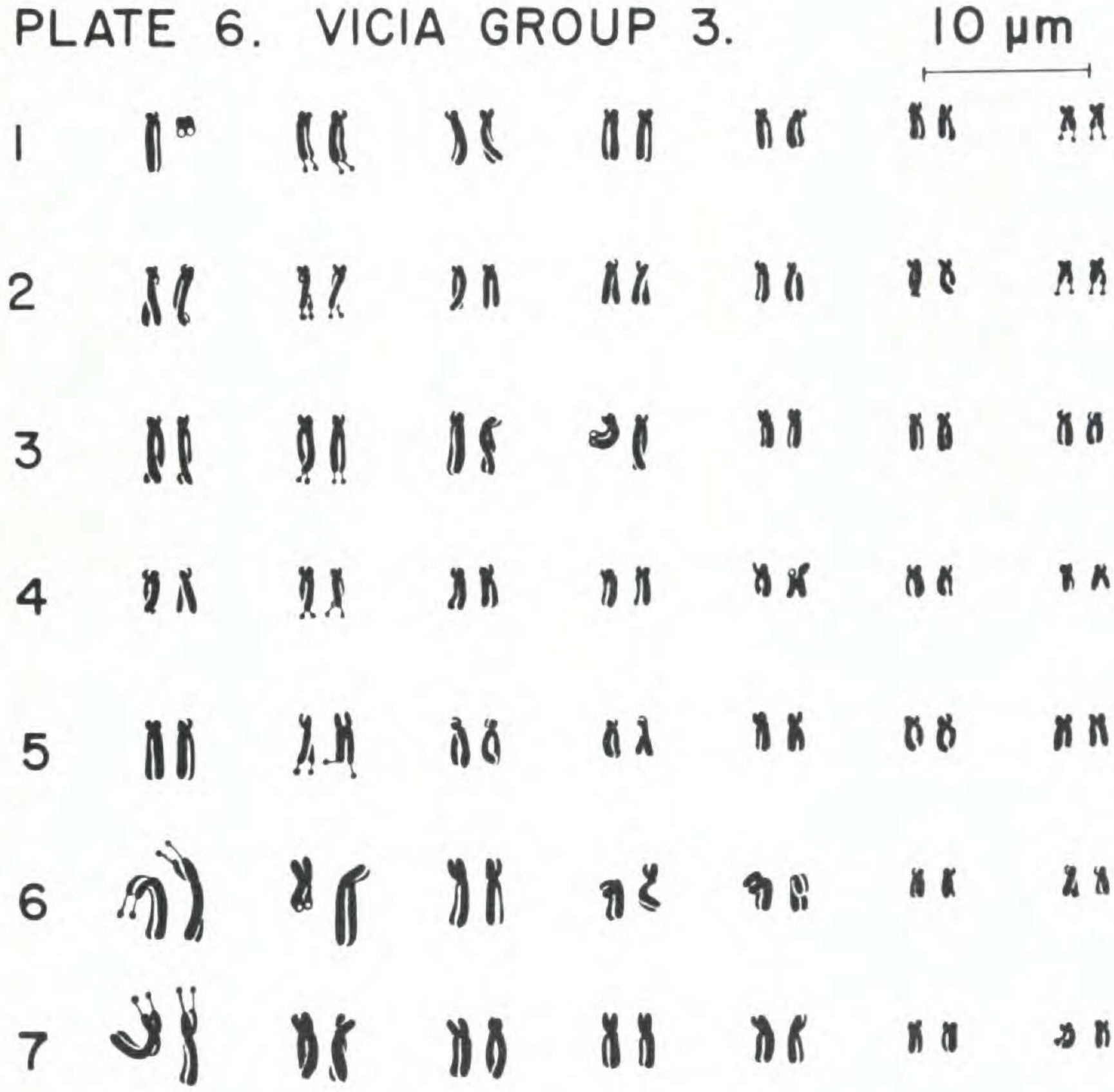

Plate 6 Figures **1–2.** *V. ocalensis* Godfrey & Kral 1. USDA *PI 316685* Marion Co., Florida, USA BARC 2. USDA *PI 316685* Marion Co., Florida, USA BARC Figures **3–5** *V. acutifolia* Ell. 3. *Lassetter 2105* Glenn Co., Georgia, USA EKU 4. USDA *PI 316683* Lake Co., Florida, USA BARC 5. *Lassetter 2138* St. Johns Co., Florida, USA EKU Figures **6 & 7.** *V. floridana* S. Wats. 6. USDA *PI 316684* Levy Co., Florida, USA BARC 7. *C. R. Gunn 3348* Levy Co., Florida, USA NA

taxonomic characters in combination with their karyotypes might justify a sub-section distinction. These two species have very different karyotypes from the species in our group three, and as stated before share karyotype characters with *V. americana* and *V. graminea*. Further work is needed to resolve this problem.

As far as we have determined from the literature, our reported number of $2n = 14$ is the first reported count for the following species: *V. acutifolia, V. caroliniana, V. floridana, V. humilis, V. minutiflora, V. nigricans* (sensu stricto), and *V. ocalensis*.

Cortazar (1948) presented a karyotype ($2n = 14$) of *Vicia macraei*

(now a synonym of *V. nigricans*, Lassetter & Gunn, 1979). For *V. americana*, $n = 7$ (Löve, 1973; Moore, 1973; Moore, 1977) and $2n = 14, 28$ (Fedorov, 1969) have been reported. Numbers of $2n = 12, 14, 28$ have been reported for *V. cracca* by Darlington and Wylie (1955), Fedorov (1969), Löve, (1973, 1975), and Moore (1973, 1977); and in addition, Fedorov (1969) reported $2n = 21, 24$. A count of $n = 7$ for *V. gigantea* (now a subspecies of *V. nigricans*, Lassetter & Gunn, 1980) was reported by Moore (1973) who also reported $2n = 14$ for *V. graminea*.

Three counts of taxa of the *Vicia ludoviciana* complex are given by Turner (1956): *V. ludoviciana* var. *typica* (race 1), $n = 7$; *V. leavenworthii* var. *typica* (race 4), $2n = 14$; and *V. leavenworthii* var. *occidentalis* (race 6), $n = 7$.

CONCLUSIONS

The karyotype groupings one and three of native New World *Vicia* presented here support Kupicha's sectional division of the subgenus *Vicilla*. The species of our group two, although similar in karyotypes, are diverse in sectional membership. Additional karyotype data, especially from South American taxa, would greatly aid in understanding the subgeneric relationships of New World vetches.

LITERATURE CITED

Bowen, C. C. 1956. Freezing by liquid carbon dioxide in making slides permanent. Stain Technology **31**: 87–90.

Cortazar, C. S. de. 1948. Observaciones cromosomales en seis especies Chilenas. Agricultura Tecnica (Chile) **8:** 28–36.

Darlington, C. D. & A. P. Wylie. 1955. Chromosome atlas of flowering plants. Hafner Pub. Co., New York. 519 pp.

Fedorov, An. A., ed. 1969. Chromosome numbers of flowering plants. V. L. Komarov Botanical Institute, Leningrad. 926 pp.

Godfrey, R. K. & R. Kral. 1958. A new species of *Vicia* (leguminosae) in Florida. Rhodora **60**: 256–258.

Gunn, C. R. 1971. Seeds of native and naturalized vetches of North America. USDA Agric. Handbook **392.** U.S. Govt. Printing Off., Washington, D. C. 42 pp.

———. 1979. Genus *Vicia* with notes about tribe Vicieae (Fabaceae) in Mexico and Central America. USDA Technical Bull. **1601.** U.S. Govt. Printing Off., Wash., D. C.

———., & J. Kluve 1976. Androecium and pistil characters for tribe Viceae (Fabaceae). Taxon **25**: 563–575.

HEITZ, E. 1931. Nukleolen und chromosomen in der gattung *Vicia*. Zeitschr. Wiss. Biol. Abt. E, Planta **15**: 495–505.

HERMANN, F. J. 1960. Vetches of the United States—native, naturalized, and cultivated. USDA Agric. Handbook **168**. U.S. Govt. Printing Off., Wash., D. C. 42 pp.

HOLMGREN, P. K. & W. KEUKEN. 1974. Index Herbariorum. Part I. The herbaria of the world. Oosthoek, Scheltema and Holkema, Utrecht.

KUPICHA, F. K. 1976. The infrageneric structure of *Vicia*. Roy. Bot. Gard. Edin. Notes **34**: 287–326.

LASSETTER, J. S. 1972. A biosystematic study of the *Vicia ludoviciana* complex (Leguminosae). Ph.D. Thesis. Iowa State Univ., Ames, Iowa. 248 pp.

———. 1975. Taxonomic status of *Vicia hassei* (Leguminosae). Madroño **23**: 73–78.

———. 1978a. Seed characters in some native North American vetches. Sida **7**: 255–263.

———. 1978b. Edaphic relationships in the *Vicia ludoviciana* complex (Leguminosae). Iowa Journal of Research **53**: 13–20.

———. (in press). Taxonomy of the *Vicia ludoviciana* complex (Leguminosae). Rhodora.

———., & C. R. GUNN. 1979. *Vicia menziesii* Sprengel (Fabaceae) rediscovered: its taxonomic relationships. Pacific Science **33**: 85–101.

LÖVE, A. 1973. IOPB chromosome number reports XL. Taxon **22**: 285–291.

———. 1975. IOPB chromosome number reports XLIX. Taxon **24**: 501–516.

METTIN, D., & P. HANELT. 1968. Bemerkungen zur Karyologie und Systematik einiger Sippen der Gattung *Vicia* L. Feddes Rept. **77**: 11–30.

MOORE, R. J. 1973. Index to plant chromosome numbers 1967–1971. Bohn, Scheltema, and Holkema, Utrecht, Netherlands. 257 pp.

———. 1977. Index to plant chromosome numbers 1973–1974. Bohn, Scheltema, and Holkema, Utrecht, Netherlands. 539 pp.

PLITMAN, U. 1967. Biosystematical study in the annual species of *Vicia* of the Middle-east. Private publication by the author. 128 pp.

ROUSI, A. 1961. Cytotaxonomical studies on *Vicia cracca* L. and *V. tenuifolia* Roth. I. Chromosome numbers and karyotype evolution. Hereditas **47**: 81–110.

SHRIVASTAVA, L. M. 1963. Cytogenetical studies in certain species of *Vicia*. Cytologia **28**: 154–169.

STANKEVICZ, A. K. 1970. On clarification of the *Vicia* L. genus systematics. Trudy Prikl. Bot. **43**: 110–125.

SVESHNIKOVA, I. N. 1927. Karyological studies on *Vicia*. Trudy Prikl. Bot. **17**: 37–72.

TURNER, B. L. 1956. The legumes of Texas. University of Texas Press, Austin.

VEERASETHAKUL, S. 1978. A preliminary survey of karyotype relationships in New World *Vicia* (Leguminosae). Master's Thesis. Eastern Kentucky University, Richmond.

———., & J. S. LASSETTER. 1979. Notes on *Vicia cracca* L. (Leguminosae-Paplionoideae) in North America. Iselya **1**: 57–62.

YAMAMOTO, K. 1973. Karyotaxonomical studies on *vicia*. I. On the karyotype and character of some annual species of *Vicia*. Japan. J. Genetics **48**: 315–327.